BCG (Bacille Calmette–Guérin) HspCs (heat-shock protein–peptide complexes) induce T-helper 1 responses and protect against live challenge in a murine aerosol challenge model of pulmonary tuberculosis

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Abstract

The need for an effective TB (tuberculosis) vaccine remains acute, with tuberculosis still one of the major killers worldwide and 3 million new infections annually. We report here on the immune responses elicited by HspCs (heat-shock protein–peptide complexes) isolated from BCG (Bacille Calmette–Guérin) vaccine. These HspCs elicit both the appropriate cellular and protective immune responses required to merit their further development as TB vaccine candidates.

Introduction

The steady rise in the incidence of TB (tuberculosis) in the developed world and the emergence of drug-resistant strains of *Mycobacterium tuberculosis* underlines the need for a more effective TB vaccine [1]. The current live BCG (Bacille Calmette–Guérin) vaccine shows highly variable efficacy in field trials, probably due to the prior exposure of vaccines to environmental mycobacteria [2]. Experimental studies suggest that this limitation of vaccine efficacy may be overcome by the use of subunit, as opposed to live, vaccines [3].

Recent advances in our understanding of antigen capture by the immune system have provided the basis for the rational design of efficacious vaccines [4]. The adjuvant properties of heat-shock proteins (Hsps) have long been known, and they have been shown to bind to a variety of cellular receptors and elicit chemokine and pro-inflammatory cytokine secretion by cells of the innate immune system [5]. However, the main role of the Hsps is that of 'molecular chaperones', binding nascent cellular proteins and assisting their correct folding [6]. Thus it should be appreciated that Hsps isolated from both prokaryotic and eukaryotic cells will invariably be complexed with the cellular proteins they are chaperoning (4). Furthermore, these HspCs (Hsp–peptide complexes) are specifically recognized by the antigen-presenting cells of the immune system, most notably the dendritic cells, which utilize the peptides complexed therein for the MHC-restricted stimulation of naive T-cells [4,7,8]. We thus proposed that HspCs may play a key role in the integration of the innate and acquired immune responses, which could provide a novel approach to vaccine development; namely, the direct use of pathogen-derived HspCs as effectively 'multi-subunit' vaccine candidates [4].

In the present study we have studied the utility of HspCs isolated from a variety of mycobacterial strains as potential vaccine candidates in the murine aerosol model of TB [9]. This model represents a physiologically relevant model system for studies of vaccine responses in TB [9,10]. The results show that vaccination with HspCs isolated from the vaccine strain BCG was just as efficacious as live BCG in protecting immunized mice against aerosol challenge with virulent *M. tuberculosis*, indicating a simple, safe, multi-subunit alternative to BCG for use in effective field vaccination campaigns.

Experimental

Immunization of mice and aerosol challenge model

Mice (6–8 weeks old, female Balb/c; Harlan, Bicester, Oxon, U.K.) were vaccinated once or twice with HspC preparations (75 μg of total protein/dose) via the subcutaneous route. BCG (Glaxo Strain) was administered once [3×10^5 CFU (colony-forming units)] via the intradermal route. After 4 weeks, mice were challenged with pathogenic *M. tuberculosis* strain H37Rv via the aerosol route, such that approx. 500 bacteria per lung were lodged. After 4 weeks post-challenge, lungs were removed, homogenized and serial-diluted samples were plated on to 7H11 agar plates. After

Key words: chaperone, heat shock, immunity, stress protein, tuberculosis, vaccine.

Abbreviations used: CFU, colony-forming units; (Hsp)C, (heat-shock protein)-peptide complex; TB, tuberculosis; WCL, whole-cell lysate.

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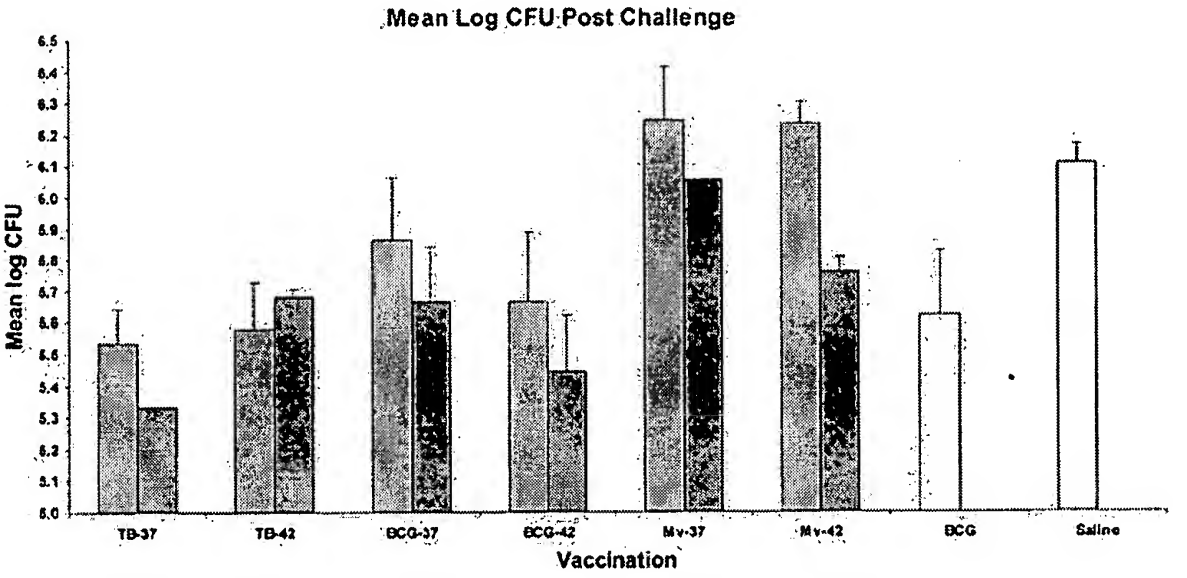
Figure 1 | Lung CFU in a standard vaccination/protection study
Protection induced by TB-37, TB-42, BCG-37, BCG-42, Mv-37 and Mv-42 vaccinations: light-grey bars 1 dose; dark-grey bars 2 doses.



2–3 weeks, viable bacterial colonies were counted to enumerate the infection as CFU.

HspC preparation and analysis

Mycobacteria were grown in 7H9 broth at 37°C and Hsps were induced at 42°C for 2 or 4 h. Preparations were spun down (2500 *g* at 4°C), resuspended in cold saline and homogenized using a ribolyser in 1.5 ml sterile tubes with glass beads. Homogenates were centrifuged and filtered through 0.45 μm and 0.2 μm filters. The protein content was determined using a Bio-Rad protein assay kit, and HspCs analysed by SDS/PAGE and Western blotting. Non-reduced samples were run on a 10% acrylamide gel and transferred on to nitrocellulose membranes. Gels were stained with Coomassie Blue, and Western blots were probed with either anti-GroEL (1:500 dilution) or anti-DnaK (1 μg/ml) and visualized using the ECL® detection system.

Cellular immune studies

Spleens were obtained from mice 4 and 45 days post-vaccination, and a lymphocyte-rich cell population was isolated using red-blood-cell lysis buffer. Cell suspensions adjusted to a density of 2×10^6 cells/ml were re-stimulated for 3 days with recombinant *M. tuberculosis* Hsp65 (10 μg/ml), *M. tuberculosis* WCL (whole-cell lysate; 10 μg/ml) or HspC preparations, and assayed for the production of interferon-γ by ELISA.

Results

The absolute amounts and ratios of the two major Hsp families, GroEL and DnaK, varied between HspC preparations isolated from the different mycobacterial species, as estimated on the basis of the Western blots (results not shown). HspC preparations containing both Hsps were assessed for the ability to reduce the numbers of viable bacteria in the lungs of mice after challenge with pathogenic *M. tuberculosis* (strain H37Rv), and the results are shown in Figure 1. The BCG HspCs provided significant protection against challenge, boosted by a second dose. In contrast, the HspC preparations from *Mycobacterium vaccae* did not appear to confer a similar degree of protection as the BCG extracts, although a booster effect was observed. Interestingly, although both the *M. tuberculosis* HspC preparations were able to confer significant protection as measured by reduced CFU in the lungs of infected mice, the booster effect was not observed with both preparations (Figure 1).

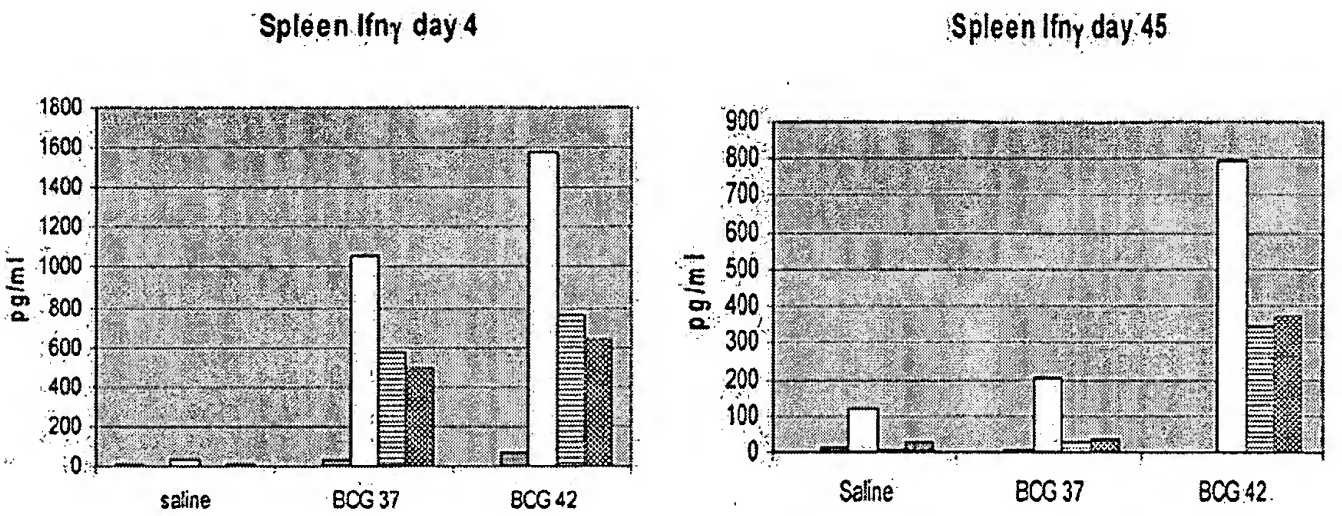
As protection induced by the live BCG vaccine is thought to be cell-mediated [10], we examined the cellular responses in animals immunized with HspC preparations for the induction of cytokines characteristic of T-cell responses. The HspC immunizations resulted in a dominance of interferon-γ responses, typical of Th1-type [10], which were induced as early as 4 days after immunization and persisted for at least 45 days, the longest time point examined (Figure 2). Although significant interferon-γ responses were observed on restimulation with the HspC preparations used for immunization, maximal responses were observed when total cell lysate from *M. tuberculosis* (WCL) was used as the re-stimulation antigen (Figure 2). In contrast, no responses were observed when recombinant Hsp65 was used (Figure 2). Consistent with their protective effect in the challenge studies (Figure 1), the higher-temperature HspC preparations induced significantly higher interferon-γ responses.

Discussion

This study has assessed mycobacterial HspCs as potential TB vaccine candidates in the mouse aerosol challenge model [9].

Figure 2 | Cell-mediated responses of mice vaccinated with BCG HspC preparations

The cell-mediated responses [shown as pg/ml interferon-γ (Ifnγ) produced] of mice vaccinated with BCG HspC preparations [either saline (control), BCG-37 or BCG-42] were investigated. The shading of the bars corresponds to restimulation with the following antigens: solid black bars, saline control; grey bars, rHsp-65; white bars, WCL; horizontal-line-shaded bars, BCG-37; and stippled bars, BCG-42.



Protective immunity was observed with a number of HspC preparations studied, most notably with the *M. tuberculosis* 37°C and BCG 42°C preparations (Figure 1). As protective immunity is thought to be cell-mediated [10], we studied the ability of BCG HspC preparations to induce cellular responses, as assayed by the induction of cytokines by spleen cells isolated from immunized mice. Consistent with the protective immunity results, the BCG HspC preparations showed a Th1-like response [10], with the induction of interferon-γ as early as 4 days after injection, which persisted for at least 45 days after immunization (Figure 2). Most interestingly, the interferon-γ response was seen in spleen cells re-stimulated with HspCs, but not the recombinant mycobacteria Hsp65 (GroEL) itself. This indicates that the cell-mediated response induced by HspC immunization is directed primarily against the pathogen-derived antigens chaperoned by the Hsp in the HspC preparation, and not the Hsp itself. This contention is supported further by the fact that the maximal induction of interferon-γ was seen on re-stimulation with TB WCLs that contain a mixture of *M. tuberculosis* antigens. These results provide proof of principle that HspC preparations from mycobacteria can provide novel and effective vaccine candidates for TB. Moreover, as the efficacy problems of the current live BCG vaccines are thought to be due to prior exposure to environmental mycobacteria, such a subunit-based vaccine approach may be more effective in the field and may even have utility as a booster/supplemental vaccine for BCG itself [1,3,11], a possibility we are now testing.

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